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The Nature and Origin of the Epidermal Scales of *Notodactylus handschini*—an Unusual Temnocephalid Turbellarian Ectosymbiotic on Crayfish from Northern Queensland

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Abstract. The temnocephalid *Notodactylus handschini*, ectosymbiotic on the crayfish *Cherax quadricarinatus* from northern Queensland, is unique among known turbellarians in having its dorsal epidermis covered by rows of closely adjacent scales. These are borne on epidermal plinths separated by arthrodial gutters and are up to 100 μm tall with rhombic bases 40–55 μm by 15–20 μm. Above the bases, the rhombic cross section gradually becomes oval so that the scales are essentially elongate conoids, the slender tips of which curve inwards towards the worm's mid-line. In mature worms, the more median scales may be reduced distally into squat truncated cones only 40–50 μm tall. The scales consist of glycoprotein; rhabdites discharged from cells in the dorsal parenchyma contribute the protein, whereas the carbohydrate component probably comes from the glycocalyxes of the epidermal microvilli. The latter act as templates around which the glycoprotein mixture coalesces, seemingly by a simple tanning process, into tightly packed tubes 180–200 nm in diameter with walls 40–45 nm thick. The scales lack any limiting wall or membrane other than a loose amorphous layer, 90–150 nm thick, formed by disintegration of the tubes distally and compensated for by continuous growth basally. Each scale is attached to its epidermal plinth by the bases of its constituent tubes ensheathing the microvilli; attachment is reinforced by cross-striated fibrils, probably collagen, embedded in the epidermis and inserted between the microvilli into tube bases near the scales' corners. Scale surfaces bear rich growths of microorganisms. The use of rhabdites to form permanent scales is probably an adaptation to the worm's unusual sedentary habit; it supports, paradoxically, an earlier hypothesis that the primary function of rhabdites in turbellarians other than temnocephalids is to provide a continuously renewable coating compatible with epidermal ciliation.

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Introduction

The epidermis in turbellarian flatworms (comprehensively reviewed by Tyler, 1984) is typically a monolayered ciliated epithelium, with microvilli, made up of distinct cuboidal, squamous, or columnar cells. It can, though, be syncytial or insunk with its nuclei and some cytoplasm lying among or even below the subepidermal musculature. The epidermis is penetrated by the necks of subepidermal glands and the dendrites of sensory structures that pass between or through the epithelial cells when these are present.

These basic patterns are remarkably constant throughout the Turbellaria and persist, for example, in those entoparasitic species that lack normal entodermal alimentary systems and use the epidermis as their sole means of nutrient uptake. In the Fecampiidae, living in the hemocoel of amphipod and isopod crustaceans, the epidermis remains typically turbellarian, and the only apparent structural modification is an increase in the density and length of the microvilli (Jägersten, 1942; Christensen and Kanneworff, 1964; Bresciani and Koie, 1970; Blair and Williams, 1987). The fecampiids take up only soluble nutrients, but even in species where the epidermis actively secretes digestive enzymes and takes up particulate material for completion of digestion intracellularly, as in the

rhabdocoel *Acholades asteris* living in the tube-feet of starfishes, the cells remain columnar, ciliated, and traversed by the necks of subepidermal glands (Jennings, 1989).

The only major departure from the typical turbellarian pattern occurs in the Temnocephalida, which are ectosymbiotes of freshwater decapod crustaceans and a few other hosts. In most temnocephalids, cilia are restricted to small areas of the tentacles or around the excretory pores, locomotion is by muscular looping using the tentacles and simple posterior sucker and not by ciliary gliding, and the syncytial epidermis is bounded distally by a narrow, clear zone of vesicular epitheliosomes (Williams, 1975, 1980, 1986). The epidermal surface still bears microvilli, though, and the syncytium is honeycombed by numerous cell necks through which subepidermal glands discharge their secretions.

The most extreme epidermal modification in the Temnocephalida and, indeed, in the Turbellaria as a whole so far as is presently known, occurs in *Notodactylus handschini*, an ectosymbiote of various crayfishes from Papua New Guinea and northern Australia. In this species, the entire dorsal surface is covered by golden-brown scales that are much taller than the underlying epidermis (Baer, 1945, 1953; Cannon, 1991). These have not yet been described in any detail, and we report here, therefore, on their nature, origin, and mode of formation, as part of a wider study on the general biology of this unusual turbellarian.

Materials and Methods

Adults, juveniles, and hatchlings of *Notodactylus handschini* (Baer 1945) (Turbellaria: Temnocephalida) were collected from the lateral margins of the carapace of the freshwater decapod crustacean *Cherax quadricarinatus* (von Martens 1868), a northern Queensland species held in culture in farm ponds near Gympie, southeast Queensland. Specimens for histological and histochemical studies were fixed in Bouin's fluid, 90% ethanol or 10% formalin buffered to pH 7.0 with 0.1 *M* sodium phosphate and used at 4°C. Paraffin wax serial sections, 4 or 8 μm thick, prepared by standard procedures, were stained by Curtis's Ponceau S method for collagen, Ehrlich's haematoxylin and eosin, Heidenhain's iron haematoxylin and metanil yellow, or Mallory's trichrome stain. Histochemical methods included an alcian blue, periodic acid-Schiff (PAS) and orange G trichrome technique for glycoproteins and mucosubstances, the mercury-bromphenol blue method for proteins, Millon's and Sakaguchi's reactions for tyrosine and arginine, Perls' method for ferrous and ferric iron, and the ammonium hydroxide-alizarin method for calcium (Pearse, 1972).

Polyphenol oxidase activity in the scales was detected by a modification of Johri and Smyth's (1956) method; formalin-fixed whole worms were treated with 0.1% aqueous catechol (1,2 benzenediol) for 1 h, sectioned in paraffin wax at 8 μm and the sections dewaxed, mounted in DPX, and examined using a deep blue filter transmitting at 350–450 nm with peak transmission at 425 nm. Controls were sections of untreated worms and whole mounts of various proseriate and digenean worms showing the vitellaria.

For ultrastructural studies, specimens were fixed for 3 h at 4°C in 3% glutaraldehyde buffered to pH 7.2 with 0.2 *M* phosphate, post-fixed for 1 h in buffered 1% osmium tetroxide, embedded in Spurr's resin, and sectioned. Thin sections, mounted on pioloform films carried on copper slot grids and stained with uranyl acetate and lead citrate, were examined in a JEOL 1200 EX transmission electron microscope. Other sections, 1–2 μm thick, were stained with toluidine blue and studied with the light microscope.

The arrangement and general topography of the scales were studied by light microscopy, using unstained formalin-fixed specimens cleared and mounted in DPX, and by scanning electron microscopy of formalin-fixed worms post-fixed in buffered 2% osmium tetroxide, processed by standard procedures and examined in a Camscan Series 3 SEM.

Results

Notodactylus handschini (Figs. 1, 2) is a broadly oval temnocephalid, 1.0–1.5 mm × 0.75–1.0 mm at maturity, with five anterior tentacles curled ventro-posteriorly when at rest, a pair of eyes anteriorly, and a well-developed sucker posteriorly. The entire dorsal epidermis is covered by golden-brown scales bearing rich growths of epizoic bacteria, cyanobacteria, diatoms, green algae, stalked ciliated protozoans, and sessile rotifers.

The scales lie in close-set rows but do not overlap; they are up to 100 μm tall with rhombic bases 50–55 μm by 15–20 μm, whose long axes lie transversely to the worm's longitudinal axis (Fig. 3). The great majority can be referred to a single basic form in which the rhombic cross-section at the base continues upwards, decreasing in area for some 15–20 μm before gradually becoming oval so that the scales are essentially elongate conoids whose slender tips curve inwards towards the worm's mid-line (Fig. 4).

Scales along the lateral body margins are always of this shape but vary in height according to their position. Those nearest the naked ventral epidermis are the smallest, rarely more than 30–35 μm tall, but the size increases across the dorsal epidermis up to 55–65 μm. In mature worms, the more median scales may be reduced distally into squat truncated conoids no more than 40–50 μm tall (Fig. 6). Their flat or slightly convex tops are covered in epizoic growths of the same variety and abundance as those colonizing other surfaces of the scales, suggesting that loss

of the curved tips is a normal consequence of aging. The smaller, lateral scales bear only light growths, restricted to their lower surfaces, supporting the conclusion that they are younger than their more dorsal counterparts.

Most scales lying along the anterior and posterior body margins are of the curved conoid type, but two or three on each of the antero- and postero-lateral margins are exceptionally tall, stout, and columnar, reaching 90–100 μm in length (Fig. 6). Their cross-sectional shapes and areas do not change along their length, and they remain, in effect, tall rhombic prisms covered on all surfaces by epizoic growths. Their tops, particularly, are prone to colonization by vorticellid ciliates. These columnar scales are especially noticeable in living worms viewed by epi-illumination, when they appear strongly iridescent.

Newly hatched *N. handschini* lack scales and are greyish-white dorsally. Scale rudiments soon appear though (Figs. 19, 20), and recognizable scales of the adult types are present within three days. These are quickly colonized by the characteristic assemblage of epizoites so that juveniles four to five days old are indistinguishable externally from adults, apart from their difference in size.

Retractile papillae, 100–150 μm by 30–40 μm when extended, occur between the rows of scales sub-anteriorly and posteriorly. They are simple outgrowths of the body wall, are devoid of epizoites, and contain muscle fibers continuous with the diagonal muscles of the general body musculature (Fig. 6). They have no connection with the scales and will not be described further here.

Histology and histochemistry of the scales, epidermis, and rhabditogen cells

Scales of all types and ages are strongly acidophilic, staining deeply with eosin, orange G, and the acid fuchsin and picric acid components of Mallory's and Curtis's stains. They also stain strongly with toluidine blue, iron haematoxylin, the mercury-bromphenol blue method for proteins, Sakaguchi's method for arginine and the PAS reaction. They stain only lightly with 1% aqueous alcian blue prior to permanganate oxidation, but more deeply subsequently, very lightly with Millon's reagent for tyrosine, and not at all with the Ponceau S component of Curtis's stain for collagen and Perls' method for inorganic iron. They react positively to the alizarin test for calcium, especially basally; the reaction is strongest in formalin-fixed scales, suggesting that the calcareous component is susceptible to the acidic constituents of Bouin's fixative. This was confirmed by treatment of formalin-fixed sections with 2% hydrochloric acid, which eliminated any subsequent response to alizarin.

Iron haematoxylin staining followed by careful differentiation in iron alum reveals darker staining bands in the basal regions of mature scales, suggestive of growth rings (Fig. 6).

The scales of formalin-fixed worms treated with 0.1% catechol prior to sectioning at 8 μm, showed a significant darkening basally when compared with scales on untreated worms, indicating the presence of polyphenol oxidase or a similar quinone-tanning enzyme system. Because even young scales are golden-brown in color, such darkening is difficult to discern with normal illumination, but using a deep blue filter with peak transmission at 425 nm, the reactive zones showed greater absorption and were clearly seen. Whole mounts and sections of various proseriate and digenean flatworms, showing vitellaria or eggshell-producing glands, acted as positive controls.

The plaque-like growths of epizoites on the scales provided useful controls for all these tests, with at least some of the various organisms showing positive reactions to one or another of them. Positive responses to Perls' test for iron were particularly common.

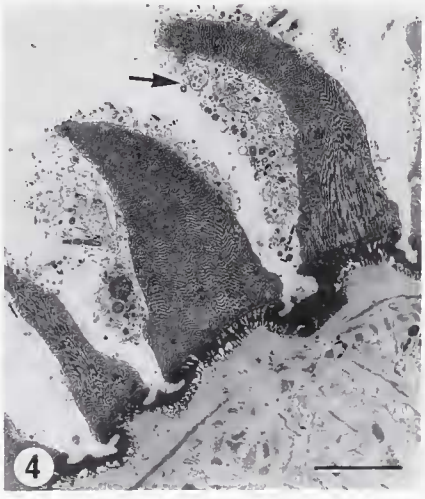
The combination of reactions shown by the scales indicates that they are glycoproteins tanned into a stable physico-chemical form by a simple quinone-tanning system. Their stability was demonstrated during the application of the Millon's test for tyrosine when they survived immersion in the reagent, containing 10% sulphuric acid, for 5 min at 60°C—a procedure that destroyed all other parts of the sections except the frustules of epizoic diatoms.

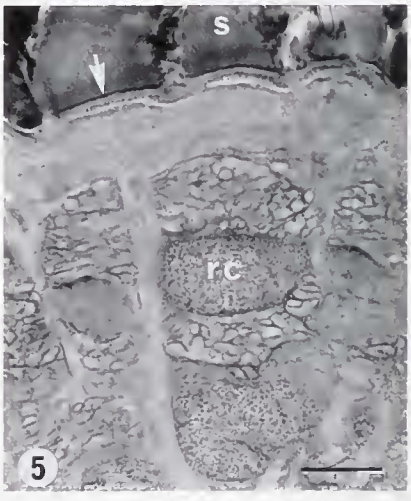
The epidermis beneath the scales is syncytial, as is that covering the rest of the body. It is 5.0–5.5 μm deep, with strongly acidophilic cytoplasm, which stains deeply with acid fuchsin, eosin, orange G, and mercury-bromphenol blue. It reacts only weakly to PAS apart from the extreme distal region, which gives a strong positive reaction (Fig. 5); this area appears as a striated border after iron haematoxylin and is obviously the microvillar layer, which is a dominant feature at the ultrastructural level (Figs. 8, 9).

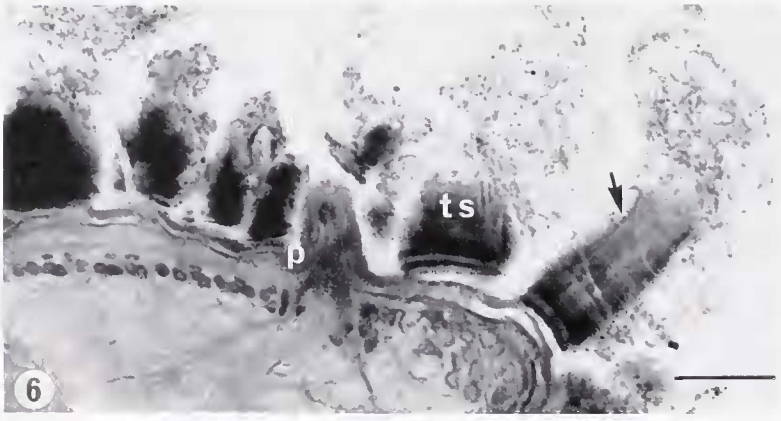
Epidermal nuclei are infrequent but prominent, 6.5–7.5 μm by 4.5–5.0 μm, lying lengthwise in the syncytium and with distinct, deeply staining chromatin. They may cause the epidermis to bulge slightly inwards, but are never insunk.

The epidermis rests on a thick fibrous basement membrane, 7.0–8.0 μm deep, which stains strongly with Curtis's Ponceau S method for collagen but only lightly with PAS.

The epidermis and basement membrane are traversed by the slender necks of rhabdite-secreting gland cells (rhabditogen cells), whose main bodies lie in the parenchyma below the dorsal subepidermal musculature (Figs. 5, 12). The rhabditogen cells occur throughout the dorsal parenchyma but are commonest anteriorly, behind the brain and above the pharynx, and posteriorly in the region of the testes. They are ovoid to spherical, 40–50 μm in diameter, with large nuclei and acidophilic cytoplasm packed with rhabdites. The latter show all the staining reactions given by the scales, including a positive response to the alizarin test for calcium. Significantly, though, they







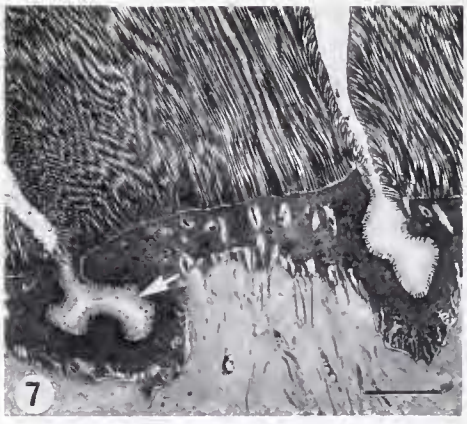


Figure 1. *Notodactylus handschini*, ventro-lateral aspect, showing the five tentacles (left), naked ventral epidermis, posterior sucker, and portions of the latero-dorsal surface covered by epizoic microorganisms growing on the epidermal scales. Some posterior scales (arrowed) bear only few epizoites. Scale bar = 200 μm.

Figure 2. Dorso-lateral aspect, showing the tentacles (right) and heavy growths of epizoites on the dorsal and lateral surfaces. Scale as in Figure 1.

Figure 3. Dorsal view of *N. handschini* photographed by dark-ground illumination after clearing and mounting unstained in DPX. The focal plane is at the level of the rhombic bases of the scales; e, eyes. Scale = 200 μm.

show no reaction to PAS and alcian blue and are extremely susceptible to mineral acids, rapidly disintegrating in the 10% sulphuric acid and 2% hydrochloric acid of Millon's and Perls' reagents. Catechol has no effect on their appearance or staining properties. The rhabdites differ from the scales, therefore, in their lack of carbohydrate and polyphenol oxidase components and solubility in mineral acids.

The cell necks of the rhabditogen cells follow a very sinuous course through the parenchyma and musculature to the epidermis and are almost impossible to trace in their entirety, even in 8 μm sections.

Ultrastructure of the scales

The scales are borne on rhombic epidermal plinths (Fig. 7), which have the same dimensions as the scales' bases. The epidermal syncytium is not noticeably thickened to form the plinths, but the plinth margins are produced into shelf-like overhangs 2.0–2.5 μm wide. These are separated from those of adjacent scales by spaces up to 5 μm wide. The epidermis dips downwards below the overhangs, emphasising the plinth-like effect, but it is turned upwards into a single fold equidistant between their tips. Each plinth is thus surrounded by a shallow gutter, about half as deep as the epidermis and separated from the adjacent gutter by the epidermal fold. The scales do not move relative to each other during the worm's normal movements, using the subepidermal musculature, and maintenance of the scales' positions is presumably due to the hinge-like action of the gutters and compensatory stretching of the epidermal folds. We suggest, therefore, the term 'arthrodial gutters' to describe these structures.

The scales are composed of ranks of uniform, closely packed parallel tubes, 180–200 nm in diameter, and with walls 40–45 nm thick (Figs. 7–11). The tubes run the length of the scales, and the majority have no visible contents; in sections cut obliquely to the scale's long axis, they may have an apparently ordered basket-weave arrangement, but examination of serial sections confirms that this is an effect of the plane of sectioning. The base of each tube encloses a single epidermal microvillus (Figs. 8, 9) but is not closely applied to it; a space 10–12 nm wide remains between the tube- and microvillar walls and is occupied by the glycocalyx. The tube bases, collectively forming the base of the scale, do not rest directly on the epidermal surface but appear to be supported some 80–90 nm above it, presumably by their connection to the microvilli via the glycocalyxes. This space was consistently present, and of the same width, in all wax and resin sections examined and would not seem, therefore, to be a shrinkage artefact. Both it and the tubes' lumina are presumably fluid-filled in life, with the fluid probably contributing significantly to the scales' mechanical stability.

The tube walls are composed of electron-opaque granules, 0.5–1.0 nm in diameter, loosely assembled into straight or slightly curved rod-shaped aggregates 20–30 nm by 8–10 nm (Figs. 9, 13, 15, 16). These tend to be orientated with their long axes at 90° to the walls' long axes. Most tubes lack visible contents, but a smaller number, 10–15% of the total, are twice the diameter of the others and are packed throughout their length with a heterogeneous mixture of granules, similar to those of the walls, and amorphous, less electron-opaque materials (Fig. 9). These larger tubes may each enclose a single microvillus basally, like the narrow tubes, or the microvilli may be lost.

Tubes forming the central bulk of the scales are straight and unbranched throughout their length. Those near the scales' edges, however, curve outwards and often branch dichotomously as they approach the edge (Figs. 10, 11). The branches are always the same diameter as the parent tube.

The scales are bounded by an unstructured layer 90–100 nm thick, which is moderately electron-opaque and formed from the disintegrating ends of the tubes. It is most distinct and uniform along straight edges of the scales near their bases (Fig. 10); it is less uniform on curved

Figure 4. Three conoid scales in vertical section. Two of the scales carry epizoic growths of various microorganisms (arrowed); the middle scale shows the transition basally from rhombic to conoid shape. Scale = 20 μm.

Figure 5. Part of a sagittal section showing the basal regions of three scales (s), the strongly PAS-positive distal border (microvillar layer) of the epidermis (arrowed), subepidermal musculature, and rhabditogen cells (rc) lying between blocks of diagonal muscles. Rhabdites in the cells are PAS-negative; their dark appearance is due to their staining with orange G. Epizoites between the scales have stained deeply with alcian blue and PAS. Section stained with alcian blue, PAS, and orange G. Scale = 20 μm.

Figure 6. Longitudinal section through the anterior region showing a tall columnar scale (arrowed) with bands, a papilla (p) whose muscle fibers extend into the parenchyma, and a truncated conoid scale (ts). Section stained with iron haematoxylin and metanil yellow. Scale = 10 μm.

Figure 7. Basal region of a conoid scale resting on its epidermal plinth, which is separated from adjacent plinths by arthrodial gutters. Microvilli lining the gutters are smaller and less regular than those at the base of the scale but bear long dense glycocalyxes (arrowed). Rhabditogen cell necks containing rhabdites are passing through the epidermal plinth. Scale = 5.0 μm.

edges, but here its origin from the walls is very obvious (Fig. 11). The layers forming the upper surfaces of the truncated scales occurring in the mid-dorsal region are of this latter type, but are usually thicker, reaching 100–150 nm, and with very disorganized lower parts. The underlying tubes, unlike those at the sides of the scales, remain straight and unbranched as they approach the surface, suggesting that the level of the latter is determined by attrition of a pre-existing curved tip.

Ultrastructure of the dorsal epidermis in relation to the scales

Dominant features of the syncytial dorsal epidermis are the tall regular microvilli of the epidermal plinths below the scales, shorter microvilli with long, dense glycocalyxes lining the arthrodial gutters, and the numerous necks of parenchymal rhabditogen and other cells which pass through it to open at the bases of the scales.

The microvilli below the scales are evenly spaced columns 1.25 μm by 0.08 μm, without internal differentiation, and with short rather granular glycocalyxes (Figs. 8, 9). Those lining the arthrodial gutters are smaller (only 0.2–0.25 μm tall), but their glycocalyxes are much larger and denser and appear as a thick fuzzy coat around the microvilli and extending above them for 0.4–0.5 mm (Fig. 7). They gradually become larger and more closely spaced along the overhanging portions of the epidermal plinths and grade into the upper surface types on the shoulder regions where scale tubes begin to form around them.

Most of the cell necks passing through the epidermis are those of rhabditogen cells lying below the subepidermal musculature in the dorsal parenchyma, whose histological and histochemical properties are described above. The cells' ultrastructure and method of rhabdite production (Fig. 12) are the same as in other turbellarians, including temnocephalids (see Smith *et al.*, 1982; Williams and Ingerfeld, 1988), and need not be described further here. Mature rhabdites leaving the cells and migrating out to the epidermis along the cell necks are elongate tapering rods, 1.50–1.75 μm by 0.20–0.25 μm, electron-opaque, and with a concentric lamellated structure (Fig. 14). They change, however, as they reach the distal epidermis; the internal lamellated structure disappears, the electron-opacity may increase or become much more heterogeneous, and they may become curved (Figs. 13, 16, 17).

The rhabdites may be retained for a time in the distal epidermis, apparently by terminal caps that seal off the cell necks (Fig. 16), but are eventually discharged onto the epidermal surface between the microvilli. On dis-

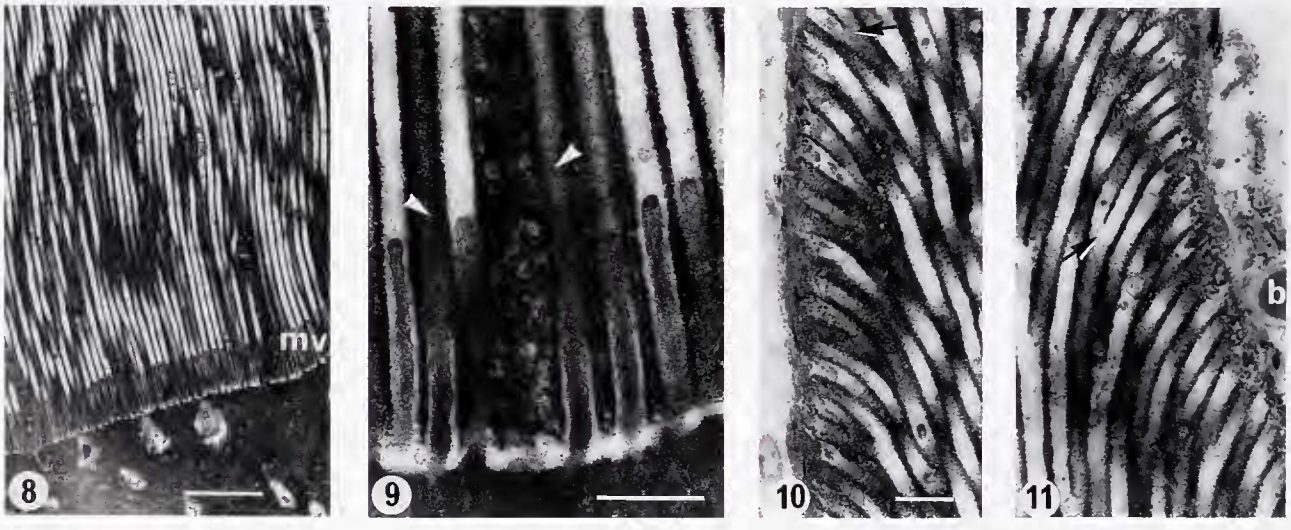


Figure 8. Part of the basal region of a conoid scale. Note the regular microvilli (mv) enclosed by the bases of the scale tubes. Rhabditogen cell necks, some containing rhabdites, are visible in the syncytial epidermis. Scale = 2.0 μm.

Figure 9. Detail from the field seen in Figure 8, showing a large tube (left of centre) whose lumen is packed with tube-wall building material. Grazing sections of walls of the commoner smaller tubes (arrowed) show the rod-shaped aggregates of wall material. Scale = 500 nm.

Figure 10. The uniformly structured layer bounding the basal region of a scale. Note the apparent branching (arrowed) of some of the scale tubes. Scale = 500 nm.

Figure 11. The curved edge of the upper part of the scale. The boundary layer is not as well organized as that shown in Figure 10. The apparent branching of scale tubes, with confluent lumina (arrowed) is clearly seen; b, epizoic bacterium. Scale as in Figure 10.

charge they disintegrate into the electron-opaque granules that form the principal components of the scale tube walls (Fig. 15). The granules are, at first, rather disorganized, but as they pass outwards between the microvilli, they become orientated into the stacked rod-shaped aggregates seen in the tube walls and in the lumina of the larger tubes (Fig. 16). During their passage outward, the aggregates themselves become automatically orientated around the microvilli to form tubes, each of which is separated from its microvillar template by the latter's glycocalyx.

The cell necks of the rhabditogen cells are 400–450 nm in diameter where they open onto the epidermal surface. They are anchored here by inconspicuous zonulae adhaerentes lying immediately above prominent septate desmosomes which encircle the necks to a depth of 450–550 nm (Figs. 13, 15, 16). They are supported internally by microtubules lying just below the cell membrane. Below the desmosomes the necks may be separated from the surrounding syncytium by apparent spaces, but these are so inconsistent in their occurrence, shapes, and sizes that they are probably shrinkage artefacts. Similar spaces occur around the cell necks where they enter the epidermis basally, and around the upward intrusions of the basement membrane into the epidermis.

Cell necks delivering rhabdites to the epidermal surface occur regularly throughout the epidermal plinths. Only occasional ones occur in the portions overhanging the arthrodial gutters, and these are curved as they divert from the main plinth out into the overhangs.

Each scale is anchored to its epidermal plinth by cross-striated fibrils that lie in cell necks opening onto the epidermal surface beneath the corners of the scale's rhombic base but inset from the overhanging portions. Each neck contains a single fibril (Figs. 17, 18); in mature worms, up to four such necks are present per corner, within a roughly circular area 1.5–2.0 μm in diameter. They are not present in hatchlings possessing only rudimentary scales but appear in juveniles, as the scales assume the adult form, within 4–5 days of hatching.

The cell necks are similar to those delivering rhabdites but are consistently larger, with neck diameters in the range 550–600 nm and with the septate desmosomes extending down into the syncytium for 600–700 nm. Unlike those of the rhabditogen cells, though, it was impossible to trace them, with any certainty, beyond the subepidermal musculature and link them with a specific cell type in the parenchyma. This was due to the absence of any identifying structural or histochemical features within the necks below the fibrils and the abundance of gland cell types in the dorsal parenchyma.

The fibrils are cylindrical, 1.5–2.0 μm long and 0.25–0.30 μm in diameter. They are provisionally identified as collagen by virtue of their characteristic appearance, being made up of regularly repeating units of dark and light bands with a periodicity of 62.04 $\pm$ 0.36 nm (n = 78, confidence limits 99%). This value was obtained from pooled data gained by direct measurement of prints and from scanning additional TEM negatives in a Fison's 'Vitatron' densitometer, normally used for scanning electrophoresis gels. It was not possible to obtain histochemical confirmation of their nature as the single fibrils could not be located in paraffin wax sections.

Each fibril lies within the cytoplasmic sheath forming the cell neck (Figs. 17, 18). Careful examination of serial sections confirmed this intracellular location; the fibrils do not lie extracellularly between parallel extensions of the cytoplasm as is usual with collagen fibrils in other animals. The cell necks are supported by microtubules, and the cytoplasm generally contains two or three mitochondria closely adjacent to the fibrils (Fig. 17). In contrast, mitochondria were never seen in the cell necks of rhabditogen cells.

The fibrils are inserted distally into the bases of the wider scale tubes that are packed with rhabdite-derived materials throughout their length (Fig. 18). They lose their regular banded structure either just within the cell neck opening or within a few nanometers of entering the scale tube and the fibril ends become frayed and dispersed into the tube contents. Proximally, the fibrils merge with the cytoplasm of the cell necks; fixation and resolution were not adequate for the details of fibril assembly to be seen.

Nothing was found to suggest that the fibrils are ciliary rootlets or the bases of sensory structures. Cilia and ciliary stubs, basal bodies, rootlets, and neuronal connections were found in the groups of sensilla on the ventral surfaces of the tentacles but nothing comparable was seen in association with the fibrils.

The cytoplasm of the syncytium is very electron-opaque and contains scattered mitochondria and profiles of cisternae. Swollen cisternae often occur alongside the cell necks (Fig. 13) but there are no indications of secretory activities into the necks or microvilli, or on to the epidermal surface.

Scale formation in young worms

Rhabditogen cells are dominant elements in the dorsal parenchyma of worms fixed 6 h after hatching, and their necks containing rhabdites are already present in the epidermis and subepidermal tissues (Fig. 12). The epidermis is syncytial and folded in a manner indicative of the future positions of the epidermal plinths. Microvilli are well-developed, especially on the upper surfaces of the folds, and simple scale rudiments may be visible around these, but most of the epidermis is naked.

Epidermal growth and folding continues and at about 12 h after hatching the future plinths and arthrodial gutters

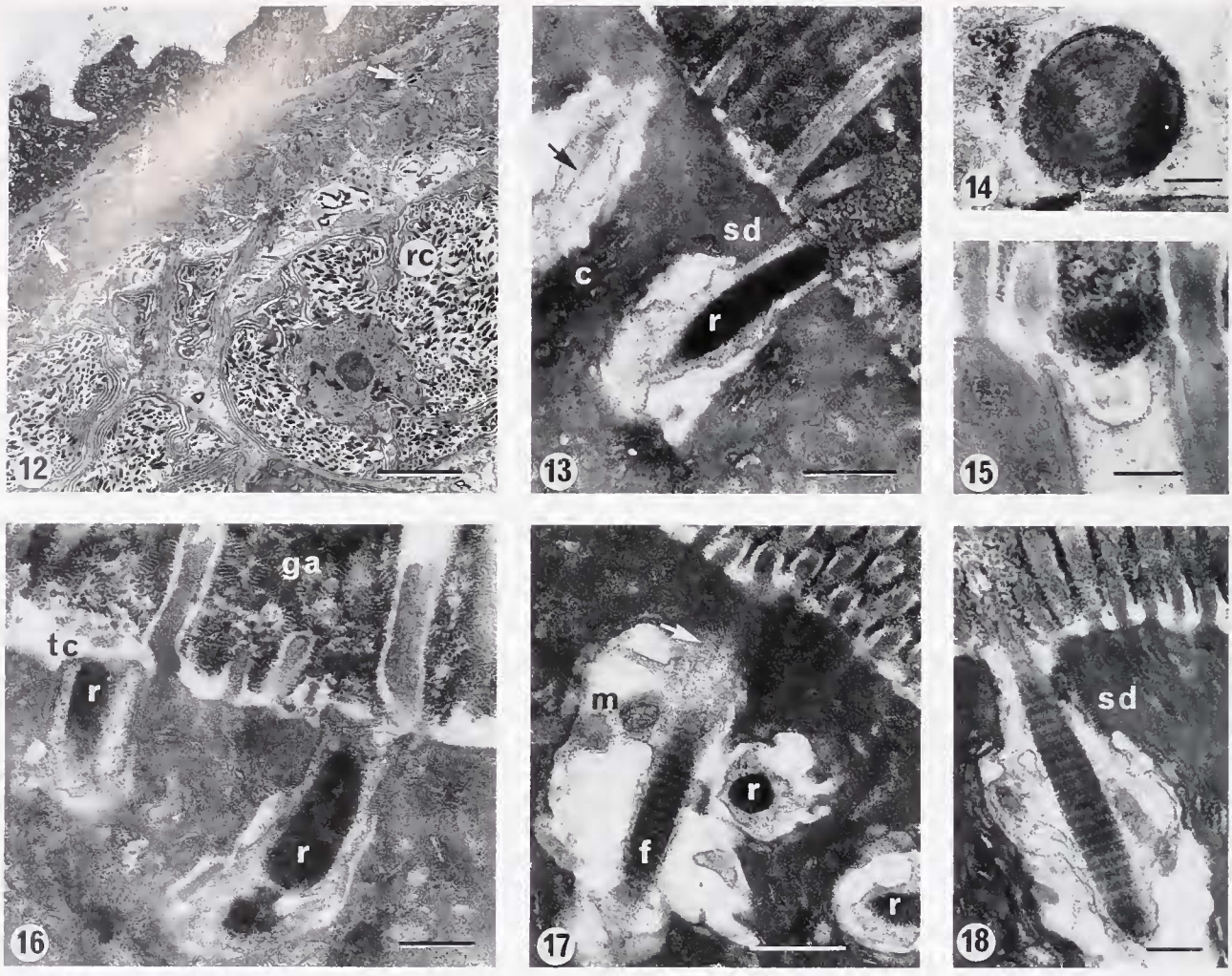


Figure 12. Part of a section from a young *N. handschini* fixed 6 h after hatching. Rhabditogen cells (rc) packed with rhabdites are prominent in the dorsal parenchyma and rhabdites (arrowed) can be seen in transit through subepidermal tissues and the epidermis. The epidermis is folded and microvilli are appearing. Scale = 5.0 μm.

Figure 13. A rhabdite (r) within a rhabditogen cell neck opening onto the epidermal surface between the microvilli. A similar cell neck containing a microtubule (arrowed) but without a rhabdite lies nearby. Note the granular aggregates, derived from discharged rhabdites, around the microvilli; c, swollen cisternae in epidermis; sd, septate desmosome. Scale = 500 nm.

Figure 14. A rhabdite in transverse section within a cell neck, showing its lamellated structure. Scale = 200 nm.

Figure 15. Remains of a discharged rhabdite lying between the bases of two microvilli. Scale = 200 nm.

Figure 16. Rod-shaped granular aggregates adding to tube bases between epidermal microvilli; ga, granular aggregates; r, rhabdites; tc, terminal cap. Scale = 300 nm.

Figure 17. Part of a striated fibril (f) embedded in the cytoplasm of a cell neck. The cytoplasm contains two mitochondria (m) and microtubules (arrowed); adjacent cell necks contain rhabdites (r). Scale = 500 nm.

Figure 18. A striated fibril in a cell neck with its distal end inserted into the base of a large tube; sd, septate desmosome. Scale = 300 nm.

are recognizable; the microvilli on the presumptive plinths are longer than those in the gutters, scale rudiments are present and many rhabdites are visible, passing through the subepidermal musculature, basement membrane and epidermis (Fig. 19).

Twenty-four hours after hatching, the basic shapes of some epidermal plinths are established, with well-defined gutters and overhangs (Fig. 20). A granular layer, up to 0.5 μm thick, is sometimes present at the level of the microvilli but disappears in older worms; it is probably

rhabdite material poured from the epidermis but not yet organized around the growing microvilli. Scale rudiments at this stage are grey and soft but can be dissected from the epidermis without losing their form, provided they are not put under excessive pressure.

Subsequent development is very rapid, and in juveniles 3–4 days old the epidermis and scales are of the adult type, with the scales' color changing from grey through pale gold to golden brown. It is at this time, significantly, that the basal regions of the scales first show a positive catechol reaction for polyphenol oxidase.

Discussion

The temnocephalid *Notodactylus handschini* is unique among known turbellarians in having its dorsal epidermis covered by precisely formed and arranged permanent scales. The only other reported occurrence of cuticular structures in the Turbellaria is in the polyclad *Enantia spinifera,* which has epidermal spines along the body margins (von Graff, 1889). The spines form as a secretion over an epidermal papilla, but the nature of the secretion and the method of its stabilization are unknown.

Despite the unique nature of the scales in *N. handschini,* their production and maintenance involve only precursors, processes and structures occurring in one form or another throughout the Turbellaria; the scales, therefore, represent exploitation of existing features rather than the evolution of entirely new ones.

The syncytial epidermis upon which the scales rest differs from that described in other temnocephalids (Williams, 1986, and references therein) in its lack of a distal layer of vesicular epitheliosomes, the presence of striated fibrils and its folding into epidermal plinths and arthrodial gutters.

The rhabdites that contribute the bulk of the scale material are of the lamellated type common elsewhere in the temnocephalids (Williams, 1975, 1986; Williams and Ingerfeld, 1988) and other turbellarians (Lentz, 1967; Bowen and Ryder, 1974; Smith *et al.,* 1982). In the temnocephalids, they disintegrate after discharge onto the epidermal surface of the tentacles to form a thin surface film which is stabilized by the microvilli (Williams, 1986). A similar constant discharge, but over the entire body surface, occurs in free-living turbellarians; the rhabdites hydrate and disintegrate to form a semi-fluid film, which is thought to protect the otherwise naked ciliated epidermis while still allowing ciliary activity (Jennings, 1957). The protective film, composed of simple, unconjugated protein, is probably constantly renewed basally as it is eroded or dissolved distally. This interpretation of the primary function of rhabdites explains why they are produced in such vast numbers, in most species, and constantly exported from their formative cells in the parenchyma into and through the epidermis. A secondary function, but still protective, is seen in polyclad turbellarians where both cotyleans and acotyleans use them to form the large gelatinous masses in which the otherwise naked eggs are embedded (Jennings, 1957).

In *Notodactylus handschini,* the protective role of the rhabdites is taken much further by elaborating them into permanent structures—the dorsal scales. Such scales, of course, are incompatible with a ciliated epidermis and ciliary locomotion but *N. handschini,* in common with most other temnocephalids, has lost most of its external ciliation and moves by muscular looping involving the tentacles and posterior sucker.

The rhabdites in *N. handschini* are very similar in histological and histochemical properties to those studied in other turbellarians by Jennings (1957), Pedersen (1959), Skaer (1961), and Bowen and Ryder (1974), and are clearly homologous with these, a view confirmed by further similarities in ultrastructure, method of secretion, and mode of export to the epidermal surface within cytoplasmic strands of the formative cells. Their involvement in scale formation, therefore, has not necessitated any basic changes in these properties. The turbellarian habit of continually discharging rhabdites through the epidermis to maintain the protective surface film lends itself readily to the formation and subsequent growth of structures like the scales of *N. handschini,* provided that the rhabdite-derived material can be stabilized. In *N. handschini,* the stabilizing factor appears to be the combination of the proteinaceous rhabdite material with a carbohydrate moiety and the subsequent tanning of the glycoprotein product by polyphenol oxidase. In view of the histochemical properties of the scales, rhabdites, and epidermis, the only possible source of this carbohydrate would seem to be the glycocalyxes of the microvilli. The polyphenol oxidase appears to be concentrated at the microvillar level, as could be expected, but its source is unknown. Its occurrence, however, is not a novel feature as it is commonly found throughout the Platyhelminthes as a tanning agent in egg capsule production (von Brand, 1973).

The occurrence in the worm's mid-dorsal region of scales that have lost their distal curved tops and become reduced to truncated conoids, shows that, despite their tanning, the scales are susceptible to erosion, perhaps by water currents or the activities of their epizoites. The constant addition of formative materials basally will compensate for this, to some extent, just as the continual discharge and disintegration of rhabdites in other species maintains indefinitely the protective film over their ciliated surfaces.

The factors determining the curved conoid shape of the majority of the scales remain unknown, along with the reasons for the occurrence of the anomalous tall columnar

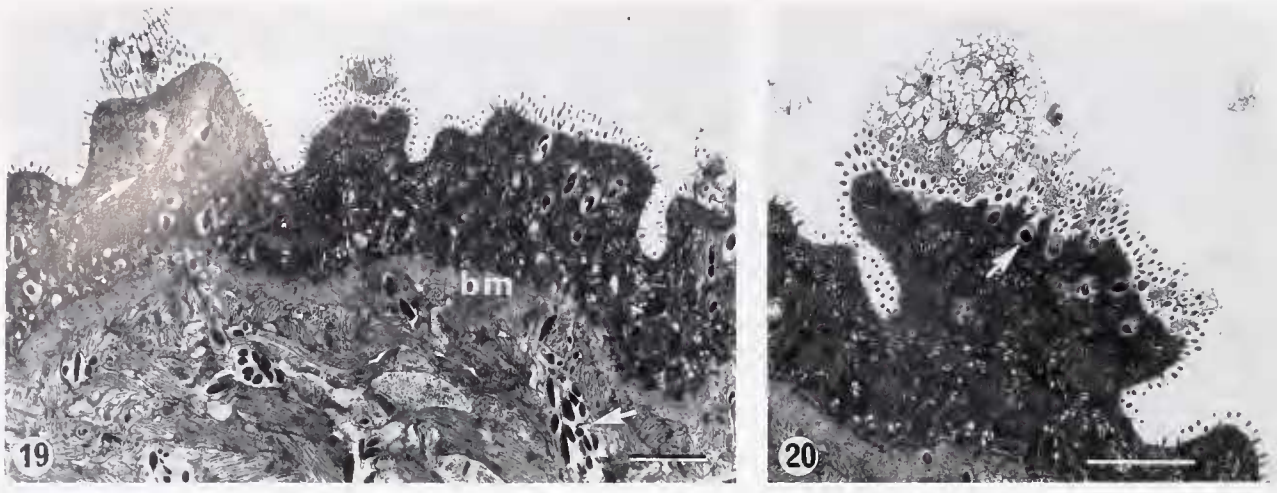


Figure 19. Part of the epidermis and subepidermal tissue of a young *N. handschini* fixed 12 h after hatching. Rhabdites (arrowed) are passing through the subepidermal musculature and folded epidermis, microvilli are well developed and scale rudiments are appearing; bm, basement membrane of epidermis. Scale = 2.0 μm.

Figure 20. An epidermal plinth and well-developed scale rudiment in a worm fixed 24 h after hatching. Rhabdites (arrowed) can be seen below the scale rudiment. Scale = 2.0 μm.

scales on the anterior and posterior body margins. Anomalous, too, is the distal branching of some of the scale tubes below the lateral surfaces of the scales (Figs. 10, 11). The branches are all the same diameter as the other tubes, suggesting, perhaps, that they result from fusion of the distal regions of adjacent tubes rather than from true branching. Alternatively, branching may occur as the tubes form around the microvilli; occasional branched microvilli do occur in young worms. If this is the case, then the distribution of microvilli, including branched ones, on the epidermal plinths is probably the decisive factor in scale morphogenesis.

Microvilli and glycocalyxes are versatile structures put to a variety of uses by animals. Examples are their roles in membrane (contact) digestion in vertebrates (Ugolev, 1965), turbellarian adhesive systems (Tyler, 1976), cuticle formation in oligochaetes and archiannelids (Potswald, 1971; Rieger and Rieger, 1976), cuticle attachment and chaeta formation in annelids (Richards, 1978), and cuticle attachment in pentastomid arthropods (Riley and Banaja, 1975). Their role in stabilizing rhabdite-derived films on the tentacular surfaces of other temnocephalids (Williams, 1986) has already been mentioned; it is not surprising, therefore, to find microvilli intimately involved in the mechanics of scale formation in *N. handschini*, additionally to the probable chemical involvement of their glycocalyxes. Their function as templates, around which the glycoprotein becomes arranged to form the scale tubes, is identical with the role of chaetoblast microvilli in the formation of chaetae from a polymerizing chitin-protein complex in annelids (Richards, 1978). In both instances, the microvilli are long and extremely regular, in accord with the long, regular tubes produced around them.

Attachment of the scales to their epidermal plinths is probably another important function of the microvilli. It is supplemented by the apparent collagen fibrils embedded in the epidermis and inserted into the bases of some of the larger tubes, which are probably strengthening pillars because they are packed with material similar to that of the tube walls.

If the striated fibrils are indeed collagen, then they are the only features associated with the scales that are novel epidermal structures; they have not been reported elsewhere in the Turbellaria. But basement membranes are usually collagenous (Burgeson, 1988), and that of *N. handschini* is probably no exception in view of its fibrous nature and PAS-and Ponceau S-positive reactions. Thus the fibril-secreting cells may well be homologous with those secreting the basement membrane and other components of the extracellular matrix.

Identification of the fibrils as collagen rests mainly upon their size, appearance, and the periodicity of their banding. The periodicity of 62.04 ± 0.36 nm lies well within the range of 55.0–68.0 nm found in examples of collagens taken from all major invertebrate groups from the Porifera to Tunicata (Baccetti, 1985). In particular, it compares with a value for *Fasciola hepatica* of 65.0 nm (Nordwig and Hayduk, 1969), which is the only other one available from the Platyhelminthes. The fibrils' location beneath the scales, their insertion into the bases of the strengthening pillars, their absence from hatchling worms possessing only rudimentary scales, and the absence of as-

sociated basal bodies, recognizable ciliary stumps, and neuronal elements (visible elsewhere in *N. handschini*) all militate against an alternative interpretation of the fibrils as ciliary rootlets. The association of mitochondria with the fibrils (Fig. 17) might be regarded as supporting this latter interpretation because mitochondria do occur alongside the rootlets of monociliated sensory processes in various turbellarians (Ehlers and Ehlers, 1977; Ferrero and Bedini, 1989). In our opinion, though, this single fact does not justify homologizing the fibrils with such rootlets, especially in view of all the other evidence to the contrary.

The fibrils are reminiscient, in position and supposed function if not in shape, of the U-shaped anchoring collagen fibrils occurring in the epidermal-dermal and basal lamina zones of vertebrates (Palade and Farquhar, 1965; Bruns, 1969; Burgeson, 1988). According to Alberts *et al.* (1989), collagen fibrils have the tensile strength of steel so that although the fibrils in *N. handschini* are relatively few in number, per scale, their concentration near the corners of the scales' rhombic bases probably does provide effective reinforcement of the scales' attachment to their epidermal plinths via the microvilli.

The supposed collagen fibrils in *N. handschini* differ in one outstanding respect from those occurring in other animals and that is their indisputably intracellular location over most of their length. There is ample and widely accepted evidence that collagen fibrils, in vertebrates at least, form by self-assembly of their constituent molecules within narrow extracellular compartments formed from parallel but separate cytoplasmic extensions of the parent fibroblasts (Birk and Trelstad, 1985, 1986; Burgeson, 1988). In *N. handschini,* the fibrils remain embedded in the cytoplasmic sheaths forming the cell necks of their formative cells and protrude from these only far enough for insertion into the bases of the larger supporting tubes of the scales. They pass through the distal neck regions, which are encircled by the septate desmosomes locking the necks into the epidermis; below this their accompanying mitochondria confirm their intracellular position.

The occurrence of scales in *N. handschini* is probably correlated with the worm's unusual life style. Other temnocephalids are very active, but this species is remarkably sedentary and remains for many days, perhaps for its entire life span, at the same location on the edge of its host's carapace (Cannon and Jennings, unpub. obs.). Mature worms surround themselves with stockade-like circles of their own egg capsules, some empty, others embryonated or newly laid, and they remain quiescent within these for long periods. They feed on small crustaceans, especially ostracods, which settle on the eggs or nearby, by rapidly extending the body, seizing the prey with their tentacles, and swallowing it intact. This behavior, of course, protects the eggs and can be construed as a form of brooding. The scales' epizoites probably facilitate feeding by concealing the worms from their potential prey; they also conceal them from potential predators while the scales themselves provide a protective shield over the body should an attack occur. *N. handschini* is not known to have any particular predators, but various other temnocepalids occur with it on the same host (Cannon, 1991), and inter-specific predation is common in such communities on other crayfishes (Jennings, 1988).

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